Annals of Clinical and Analytical Medicine
Original Research

Investigation of the effect of fuziline on end-organs in balb-c mice with cardiac damage caused by high dose dobutamine

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Abstract

Aim: Inotropes cause an increase in cardiac output because they increase cardiac contractility through different mechanisms of action. However, they also have vasodilatory or vasoconstrictive effects that vary depending on the substance and dose. In this study, we histopathologically investigated the level of damage in end-organ tissues and the effect of fuziline on these organs in mice with cardiac injury induced by high doses of dobutamine.

Material and Methods: Thirty-two adult male Balb-c mice weighing approximately 18-20 g were included in the study. They were randomly divided into four groups (n=8): Group 1 (sham, n=8), Group 2 (dobutamine, n=8), Group 3 (dobutamine + fuziline, n=8) and Group 4 (fuziline, n=8). CK-MB as a biochemical parameter for damage assessment was analyzed in all groups. Histopathological examination was performed for the presence of necrosis, inflammation and edema in the tissues of the end-organs.

Results: CK-MB was found statistically significant between the groups ($p<0.05$). When the end-organ tissues of all groups were histopathologically compared for the presence of necrosis, inflammation and edema, no significant difference was observed and no damage was detected.

Discussion: Dobutamine, which is frequently used as a positive inotropic agent in cardiovascular surgery, was observed not to cause any damage to the end-organs. In addition, we believe that the absence of any tissue damage in mice in the group given only fuziline demonstrates the safety of fuziline.

Keywords

Cardiac Damage, Fuziline, Dobutamine, Antioxidant

DOI: 10.4328/ACAM.21840 Received: 2023-08-02 Accepted: 2023-09-06 Published Online: 2023-09-19 Printed: 2023-12-01 Ann Clin Anal Med 2023;14(12):1115-1119
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This study was approved by the Local Ethics Committee of Harran University (Date: 2023-03-10, No: 01-09)

Introduction

High blood pressure, high cholesterol and smoking play an important role in the formation of cardiovascular diseases (CVD). Despite this, the main factor is oxidative stress [1]. Oxidative stress results from the deterioration of the balance between oxidant-antioxidant in favor of oxidants. CVD, causes DNA damage, neuro-degenerative and the formation of disorders such as diabetes [2]. CVD includes peripheral artery disease, coronary heart disease, rheumatic heart disease, etc. [3]. The use of inotropes is recommended in cases of end-organ dysfunction caused by hypoperfusion in patients with CVDs [4]. Dobutamine, preferred as a positive inotropic agent, activates α1 and β1 receptors to increase cardiac output and regulate mean arterial pressure [5]. However, care should be taken in dose adjustment. Therefore, an overdose may cause the development of tachycardia [6]. Today, safer and more cost-effective alternatives are being investigated in the treatment of CVDs. In this respect, medicinal plants are of great importance [7]. Among these medicinal plants, those with components such as flavonoids, carotenoids and polyphenols have been shown to inhibit CVD [8].

Flavonoids are beneficial compounds with cardiovascular, antioxidant, anti-inflammatory etc. activities [9]. Fuzi, contains compounds such as flavonoids and alkaloids in its chemical content [10]. Fuziline is one of the components of fuzi, and in terms of side effects, fuzi's other components, mesaconite, aconite and hypaconite, have fewer side effects than diterpenoids. It is safer [11].

In our study, we aimed to investigate histopathologically the level of end-organ damage (lung, liver, kidney and spleen) tissues and the effect of fuziline on these organs in mice with cardiac injury induced by high dose of dobutamine.

Material and Methods

Ethical Approval

Our study was conducted with the scientific committee approval of Harran University Animal Experiments Local Ethics Committee, dated 10-03-2023, session numbered 2023/001, and with decision 01-09.

Creation of Animal Groups and Damage Model

For our study, 32 adult male mice of the balb-c genus weighing 18-20 grams (gr) were included in the study. They were randomly (n=8) divided into four groups. In the cages created in such a way that the transparent feed-water attachments that can be seen inside can be reinforced, they were maintained in a dark environment with a relative humidity of 50%, where the temperature was 22 ±2 0 C, 12 hours of light and 12 hours of darkness. Mice were fed under standard conditions. Group 1 (Sham, n=8) was fed standard mouse chow and mains water for 15 days. Group 2 (dobutamine, n=8) were injected mice intraperitoneally (IP) with 40 micrograms (μg)/mouse/day of dobutamine for 15 days. Group 3 mice were injected with dobutamine + fuziline, n=8. For the first week mice were administered dobutamine 40 μg/mouse/day as IP. For the next week, in addition to dobutamine, 3 milligrams/kilogram (mg/kg) of fuziline was administered IP every day. Group 4 (fuziline, n=8) mice were injected with 3 mg/kg of fuziline IP for 15 days. One mouse from the fuziline group died on the eighth day. On the eighth day, all groups underwent). After detecting damage in the dobutamine+fuziline group, fuziline injection was started. The total working time was 15 days. Feeding of 31 mice was interrupted 8 hours before sacrifice. At the end of the experimental period (Day 16), all mice were sacrificed under deep anesthesia (Ketamine 90 mg/kg and xylazine 10 mg/kg-IP). Blood samples and end organ tissues were collected.

Preparation of Dobutamine

Sigma brand dobutamine (250 mg) was used to induce cardiac injury. 1.6 ml dobutamine was completed to 100 ml with saline. Each mouse was injected with 0.1 ml of the IP route each day.

Preparation of Fuziline

Sigma brand fuziline was purchased from the distributor of Interlab company in Turkey. Fuziline (0.96 mg/kg) was dissolved in 1.6 ml of dimethyl sulfoxide (DMSO). Each mouse was injected with 0.1 ml IP every day.

CK-MB (Creatine kinase myocardial band) study preparation

Blood collected from the mice hearts was transferred into biochemistry tubes with yellow caps. Centrifugation was performed at 4000 rpm for 10 minutes. The resulting plasma was transferred to Eppendorf tubes and stored at -80 ° C until the working day. During the working day, the Ependorfs were thawed at room temperature. The CK-MB value was measured with the Atellica Siemens device and the Atellica Siemens® commercial kit.

Histopathological Examination of End-Organ Tissues

End-organ tissue samples from mice were detected by placing them in 10% formaldehyde. The appropriate samples were embedded in paraffin after four hours of tissue monitoring in a Leica Bond-Max Immunohistochemistry tissue tracker. Four micrometer (μm) thick sections were obtained from the end-organs of each mouse and stained with hemotoxylin eosin (He) stain. Histopathological (Hex400) examination examined the presence of necrosis, inflammation and edema in end-organ tissues.

Statistical analysis

SPSS Windows version 24.0 package program was used for statistical analysis and $p<0.05$ was considered statistically significant. The suitability of the data for a normal distribution was tested by the Kolmogorov-Smirnov and Shapiro-Wilk tests. Comparisons between the groups were analyzed using ANOVA (one-way analysis of variance) test and Post-Hoc multiple comparisons (Bonferroni test) in the studied parameter. As descriptive statistics, mean±standard deviation was used for numerical variables and numbers and percentages were used for categorical variables.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

When CK-MB (ng/mL) levels were compared between groups, there was a significant correlation between Sham and Dobutamine ($p=0.000$), Dobutamine and Fuziline ($p=0.000$), Dobutamine+Fuziline and Dobutamine groups ($p=0.04$). It was found statistically significant ($p<0.05$) (Table 1).

When all groups were compared histopathologically in terms of necrosis, inflammation and edema, no significant difference was observed. In the histopathological examination of end-

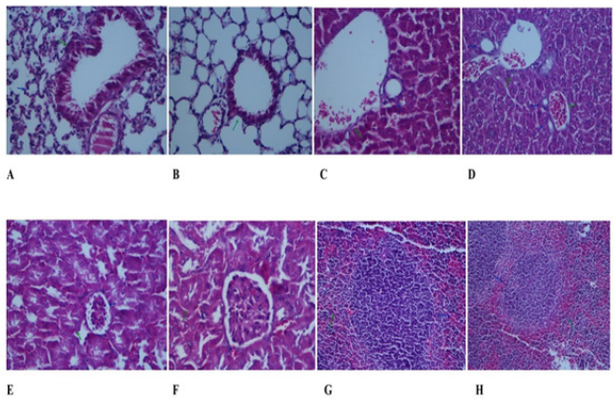

Figure 1. A, Dobutamine group lung alveoli. B, Dobutamine+fuziline group lung alveoli. C, Dobutamine group liver tissue. D, Dobutamine+fuziline group liver tissue. E, Dobutamine group kidney tissue section. F, Dobutamine+fuziline group kidney tissue. G, Dobutamine group spleen tissue. H, Dobutamine+fuziline group spleen tissue (HEX400).

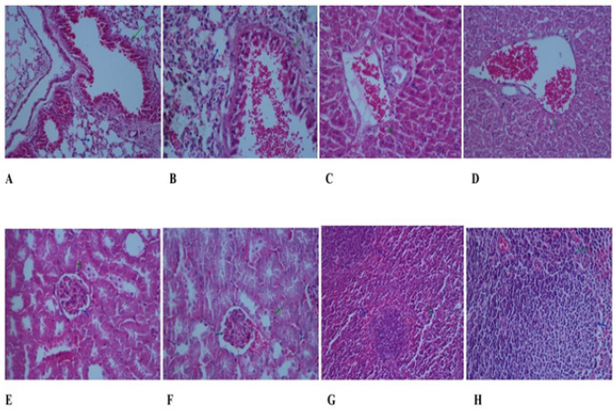

Figure 2. A, Sham group lung alveoli. B, Fuziline group lung alveoli. C, Sham group liver tissue. D, Fuziline group liver tissue. E, Sham group kidney tissue section. F, Fuziline group kidney tissue. G, Sham group spleen tissue. H, Fuziline group spleen tissue (HEX400).

organ tissues of dobutamine-injected and dobutamine+fuziline groups, regular alveoli (blue arrow) and bronchial (green arrow) structures were observed in all lung tissues of both groups (Figures 1A and 1B).

Histopathological examination of the liver tissue showed regular structure in portal areas, hepatic veins and hepatocytes (Bile duct blue arrow and congestive vascular structure green arrow, Figures 1C and 1D).

When all the right and left kidney tissues were examined, a regular structure was observed in the glomerular structures, kidney tubules and pelvicalyceal system (glomerulus blue arrow and tubule green arrow, Figures 1E and 1F).

On the histopathological examination of the spleen tissue, regular red pulp (green arrow) and white pulp (blue arrow) structures were observed (Figures 1G and 1H).

In addition, as a result of the histopathological examination of end-organ tissues of the sham and fuziline groups no damage was found in the lung (Figures 2A and 2B), liver (Figures 2C and 2D), kidney (Figures 2E and 2F) and spleen (Figures 2G and 2H).

In the histopathological examination of the end-organ tissues of 31 mice belonging to four groups, no necrosis, inflammation and edema were detected in all tissues.

Discussion

Inotropes are agents that increase cardiac output in various ways. They show different activities depending on the substance and dose. In general, they have a positive effect on hemodynamics and regulation of symptoms. Therefore, their use in patients with acute and advanced heart failure (HF) is critical. Despite these positive effects, they can also lead to increased mortality due to side effects and misuse. Dobutamine is preferred as a positive inotropic agent to eliminate blockages, despite the possibility of reducing survival in patients with HF [12]. When using high doses [10 µg/kg/minute (min)], it shows inotropic and chronotropic activity; at low doses (5µg/kg/min), it causes mild vasodilator and inotropic function that contributes

Table 1. Statistical Analysis of CK-MB in Groups.

Bonferroni						
(I) GROUPS	(J) GROUPS	Mean Difference (I-J)	Std. Error	Sig.	99,2% Confidence Interval	
					Lower Bound	Upper Bound
Sham	Dobutamine	-,29000*	0,05341	0	-0,4804	-0,0996
	Dobutamine+Fuziline	-0,08625	0,05341	0,705	-0,2766	0,1041
	Fuziline	0,00875	0,05341	1	-0,1816	0,1991
Dobutamine	Sham	,29000*	0,05341	0	0,0996	0,4804
	Dobutamine+Fuziline	,20375*	0,05341	0,004	0,0134	0,3941
	Fuziline	,29875*	0,05341	0	0,1084	0,4891
Dobutamine+Fuziline	Sham	0,08625	0,05341	0,705	-0,1041	0,2766
	Dobutamine	-,20375*	0,05341	0,004	-0,3941	-0,0134
	Fuziline	0,095	0,05341	0,517	-0,0954	0,2854
Fuziline	Sham	-0,00875	0,05341	1	-0,1991	0,1816
	Dobutamine	-,29875*	0,05341	0	-0,4891	-0,1084
	Dobutamine + Fuziline	-0,095	0,05341	0,517	-0,2854	0,0954

Tested using ANOVA (one-way analysis of variance) and Post Hoc multiple comparisons (Bonferroni test), *p < 0.05 is significant. Sham (n=8), Dobutamine (n=8), Dobutamine+Fuziline (n=8), Fuziline (n=7).

to the development of hypotension [13]. Tang et al. examined the effect of long-term use of dobutamine (10.0 μg/kg) on septic myocardial dysfunction and injury by performing cecal ligation and puncture in rats. While the probability of survival in rats with dobutamine is positively affected, no effect was observed on maintaining low levels of myocardial dysfunction and myocardial injury after sepsis [14]. In a study of female rats, dobutamine was administered to female rats at doses of 5, 10, and 20 ug/min/kg at weeks 1 and 6 before and after spinal cord injuries above T6. As a result of the study, an increase in heart rate, ejection fraction and cardiac output was observed with dobutamine infusion before spinal cord injuries above T6, while a decrease in end-diastolic volume was observed. Spinal cord injuries above T6 were followed by dobutamine supplementation at 6 weeks, which led to an increase in heart rate, ejection fraction and cardiac output, as well as an increase in pulse volume [15]. In their study of rats with and without spinal cord injury, Fernandes and colleagues administered dobutamine at different doses such as 20, 50 and 100 μg/kg. Rats with spinal cord injury had reduced their struggle with cardiac stress and increased the incidence of arrhythmias with increasing doses of dobutamine [16]. In the study on female pigs, 1, 3, 10 and 30 μg/kg/min were given as continuous infusions without dobutamine loading dose. Dobutamine was announced to increase right ventricular pressure and function, but at the expense of increased mechanical work at the highest doses, exhibiting an unfavorable hemodynamic profile [17]. In their study, Mert et al. administered dobutamine at doses of 5, 10, and 15 μg/kg/min in a total of 73 patients with HF. It has been observed that ventricular arrhythmias are triggered in this way. Even, 4 patients were excluded from the study because they could not handle high doses of dobutamine administration [18]. In our previous study with dobutamine, mice were given a dobutamine dose of 40 μg/mouse/day for fifteen days. This dose appeared to cause cardiac damage [19].

According to the data of studies conducted in recent years, it has been revealed that the use of phytochemicals is the right approach to prevent CVD [20]. Fuzi, thanks to compounds such as flavonoids and alkaloids in its chemical content [10] , stands out with its protective feature against CVD by showing anti-oxidative [21] and anti-inflammatory [22] activities.

The derivation of Fuzi [21] and the polysaccharides obtained from the purified part FZPS-1 exhibit antioxidant effects by the elimination of superoxide radical, hydroxyl radical and nitrite from the environment [22]. Fuziline and some other diterpenoids were used in the study on mice for the treatment of CVD. Zhao et al.'s investigations involving fuziline, mesaconite, neolin, and hypoconite have proven that these diterpenoids exert significant analgesic effects. In terms of harmful effects, fuziline has been observed to have fewer side effects than other diterpenoids. In addition, the maximum dose of fuziline administration was stated as 1 g/kg [11]. In the study investigating the protective efficacy and mechanisms of fuziline on in vitro and in vivo myocardial injury, the protection of fuziline against myocardial injury has been clearly demonstrated and it has been suggested that it can be used as a therapeutic agent [23].

Limitation

According to our literature review, the lack of studies examining the effect of dobutamine on end organs in terms of dose and duration is our limitation.

Conclusion

According to our literature review, the effect of dobutamine on end organs has not been investigated histopathologically. In our study, CK-MB level was examined as a result of high dose injection of dobutamine, which is frequently used as a positive inotropic agent in cardiovascular surgery, and it was determined that cardiac damage occurred in dobutamine and dobutamine+fuziline groups. However, when the last organ tissues were examined histopathologically, no damage was detected. In addition, we believe that the absence of any tissue damage in the mice in the group given only fuziline demonstrates the safety of fuziline.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Funding: *None*

Conflict of interest

The authors declare no conflict of interest.

References

1. van der Pol A, van Gilst W, Voors AA, van der Meer P. Treating oxidative stress in heart failure: past, present and future. Eur J Heart Fail. 2019;21(4):425-35.

2. Hajam YA, Rani R, Ganie SY, Sheikh TA, Javaid D, Qadri SS, et al. Oxidative Stress in Human Pathology and Aging: Molecular Mechanisms and Perspectives. Cells. 2022;11(3):552.

3. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carsonet AP, et al. Heart disease and stroke statistics-2019 update: a report from the American Heart Association. Circulation. 2019;139(10):e56-528.

4. Lindenfeld J, Albert NM, Boehmer JP, Collins SP. Ezekowitz JA, Givertz MM, et al. Heart Failure Society of America. HFSA 2010 comprehensive heart failure practice guideline. J Card Fail. 2010;16(6):e1-194.

5. Brener MI, Rosenblum HR, Burkhoff D. Pathophysiology and advanced hemodynamic assessment of cardiogenic shock. Methodist Debakey Cardiovasc J. 2020;16(1):7-15.

6. Kislitsina ON, Rich JD, Wilcox JE, Pham DT, Churyla A, Vorovich EB, et al. Shock -classification and pathophysiological principles of therapeutics. Curr Cardiol Rev. 2019;15(2):102-13.

7. Shaito A, Thuan DTB, Phu HT, Nguyen THD, Hasan H, Halabi S, et al. Herbal Medicine for Cardiovascular Diseases: Efficacy, Mechanisms, and Safety. Front Pharmacol. 2020;11:422.

8. Vasanthi H, Shrishrimal N, Das D. Phytochemicals from plants to combat cardiovascular disease. Current Medicinal Chemistry. 2012;19(14):2242-51.

9. Jucá MM, Filho FMSC, Almeida JCd, Mesquita DdS, Barriga JRdM, Dias KCF, et al. Flavonoids biological activities and therapeutic potential. Nat Prod Res. 2020;34(5):692-705.

10. Zhou G, Tang L, Zhou X, Wang T, Kou Z, Wang Z. A review on phytochemistry and pharmacological activities of the processed lateral root of Aconitum carmichaelii Debeaux. J Ethnopharmacol. 2015;160:173-93.

11. Zhao D, Shi Y, Zhu X, Cui R, Yang W, Li B. Identification of Potential Biomarkers from Aconitum carmichaelii, a Traditional Chinese Medicine, Using a Metabolomic Approach. Planta Medica. 2018;84(6–07):434-41.

12. Farmakis D, Agostoni P, Baholli L, Bautin A, Comin-Colet J, Crespo-Leiro MG, et al. A pragmatic approach to the use of inotropes for the management of acute and advanced heart failure: an expert panel consensus. Int J Cardiol. 2019;297:83-90.

13. Levy B, Clere-Jehl, R, Legras A, Morichau-Beauchant T, Leone M, Frederique G, et al. Epinephrine Versus Norepinephrine for Cardiogenic Shock After Acute Myocardial Infarction. J Am Coll Cardiol. 2018;72(2):173-82.

14. Tang X, Xu Y, Dai X, Xing Y, Yang D, Huang Q, et al. The Long-term Effect of Dobutamine on Intrinsic Myocardial Function and Myocardial Injury in Septic Rats with Myocardial Dysfunction. Shock. 2021;56(4):582-92.

15. DeVeau KM, Martin EK, King NT, Shum-Siu A, Keller BB, West CR, et al. Challenging cardiac function post-spinal cord injury with dobutamine. Auton Neurosci. 2018;209:19-24.

16. Fernandes S, Oatman E, Weinberger J, Dixon A, Osei-Owusu P, Hou S. The susceptibility of cardiac arrhythmias after spinal cord crush injury in rats. Exp Neurol. 2022;357:114200.
17. Lyhne MD, Dragsbaek SJ, Hansen JV, Schultz JG, Andersen A, Nielsen-Kudsk JE. Levosimendan, milrinone, and dobutamine in experimental acute pulmonary embolism. Pulm Circ. 2021;11(3):20458940211022977. DOI: 10.1177/20458940211022977.
18. Mert KU, Mert GÖ, Morrad B, Tahmazov S, Mutlu F, Çavuşoglu Y, et al. Effects of ivabradine and beta-blocker therapy on dobutamine-induced ventricular arrhythmias. Kardiol Pol. 2017;75(8):786-93.
19. Hacanli Y, Aydin MS, Ersöz EK, Kankiliç N, Koyuncu İ, Güldür ME, et al. Protective Effects of Fuziline on DobutamineInduced Heart Damage in Mice. Braz J Cardiovasc Surg. 2023;38(4):e20220251.
20. Islam SUl, Ahmed MB, Ahsan H, Lee YS. Recent Molecular Mechanisms and Beneficial Effects of Phytochemicals and Plant-Based Whole Foods in Reducing LDL-C and Preventing Cardiovascular Disease. Antioxidants (Basel). 2021;10(5):784.
21. Zhang XL. Enzyme-assisted extraction of aconite polysaccharide and its antioxidant activity in vitro. Zhejiang J. Trad. Chin. Med. 2016;51:912-13.
22. Yang X, Wu Y, Zhang C, Fu S, Zhang J, Fu C. Extraction, structural characterization, and immunoregulatory effect of a polysaccharide fraction from Radix Aconiti Lateralis Preparata (Fuzi). Int J Biol Macromol. 2020;143:314-24.
23. Fan CL, Yao ZH, Ye MN, Fu LL, Zhu GN, Dai Y, et al. Fuziline alleviates isoproterenol-induced myocardial injury by inhibiting ROS-triggered endoplasmic reticulum stress via PERK/eIF2alpha/ATF4/Chop pathway. J Cell Mol Med. 2020;24(2):1332-44.

How to cite this article:
Yasemin Hacanlı, Ezhar Korkmaz Ersöz, Mehmet Salih Aydın, Muhammet Emin Güldür, Reşat Dikme, Mahmut Padak, Evren Büyükfirat. *Investigation of the effect of fuziline on end-organs in balb-c mice with cardiac damage caused by high dose dobutamine. Ann Clin Anal Med 2023;14(12):1115-1119*

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